

Ultrasensitive detection of waste products in water using fluorescence emission cavity-enhanced spectroscopy

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Clean water is paramount to human health. In this article, we present a technique for detection of trace amounts of human or animal waste products in water using fluorescence emission cavity-enhanced spectroscopy. The detection of femtomolar concentrations of urobilin, a metabolic byproduct of heme metabolism that is excreted in both human and animal waste in water, was achieved through the use of an integrating cavity. This technique could allow for real-time assessment of water quality without the need for expensive laboratory equipment.

water contamination | fluorescence spectroscopy | femtomolar detection

It is axiomatic that the quality of water is essential for human health (1). The increasing worldwide contamination of freshwater systems with thousands of industrial and natural chemical compounds is one of the key environmental problems facing humanity today, where pathogens in water cause more than 2 million deaths annually (2). With more than one-third of the accessible and renewable freshwater used for industrial, agricultural, and domestic applications, pollution from these activities leaves water sources contaminated with numerous synthetic and geogenic compounds (2, 3). In addition, natural disasters can result in large-scale disruptions of infrastructure, resulting in compromised water quality. Diarrheal disease caused from such disasters may be a major contributor to overall morbidity and mortality rates (4). Thus, the cleanliness and safety of public water sources has prompted researchers to look for rapid and sensitive indicators of water quality. Whereas most water filtering systems are quite efficient in removing large-size contaminants, smaller particles frequently pass through. These contaminants are often poorly soluble in water, thus, present in quantities of less than 1 nM. Here, we demonstrate femtomolar detection of urobilin, a biomarker found in human and animal waste in water.

Modern analytical tools have become extremely efficient in the detection and analysis of chemical compounds. For example, liquid chromatography coupled with detection by tandem mass spectrometry has been commonly used for detection of trace pharmaceuticals and other wastewater-derived micropollutants (5). Although such methods are very powerful in identifying trace pollutants, cost prohibits their widespread use by environmental researchers and, most importantly, prevents real-time analysis of water quality (6). Other techniques using bench top gas chromatography–mass spectrometry have also been demonstrated as viable methods for detection of basic pharmaceuticals with reduced cost (7). Despite this, these methods are still cost prohibitive, can hardly be used in field studies, and are unlikely to ever be used for real-time quality control.

In addition to pharmaceutical and other synthetic pollutants such as pesticides, animal and human waste (i.e., feces, urine) is an enormous source of water contamination that can be found in both recreational and source waters. These discarded products, when released into water, can carry a variety of diseases such as polio, typhoid, and cholera (8). In extreme cases pollution of an

ecosystem can result in environmental crises, such as devastation to the aquatic population, red-tide blooms, as well as beach closings. Molecular methods based on polymerase chain reactions are commonly used to monitor viral, bacterial, and protozoan pathogens in wastewater (9). Microbiological indicators such as fecal coliforms, *Escherichia coli* and *Etherococci*, are the indicators most commonly used to analyze and evaluate the level of fecal contamination. However, the suitability of these indicators has been questioned (10), and it takes a substantial time from the extraction of water sample for analysis to the moment when the results are ready.

An alternative indicator that has been shown to be helpful in detection of waste in water supplies is urobilin (11). Urobilin is one of the final byproducts of hemoglobin metabolism, and is excreted in both the urine and feces of many mammals, including humans and common livestock (cows, horses, and pigs) (12). In addition, as urobilin can be indicative of disease such as hepatic dysfunction, or jaundice, an ultrasensitive technique for detection and quantification of this biomarker in solution has both diagnostic and environmental applications.

Urobilin detection in solution has previously been demonstrated using the formation of a phosphor group from the combination of urobilins and zinc ions (13). Normal heme catabolism results in the production of bilirubin, a red product, which is then broken down into two end products, stercobilin, the bile pigment found in fecal material, and urobilin, the yellow pigment found in urine. Both urobilin and stercobilin have been shown to be viable biomarkers for detection of fecal pollution levels in rivers (14).

Fluorescent detection of urobilin in urine has been demonstrated based on Schlesinger's reaction in which an urobilinogen–zinc chelation complex exhibits a characteristic green fluorescence when excited by blue light (15). Methods for detection of urobilinoids using high-performance liquid chromatography with a reversed-phase column and an ultraviolet detector have also been

Significance

Clean water is paramount to human health. Contaminants, such as human waste products in drinking water, can result in significant health issues. In this article, we present a technique for detection of trace amounts of human or animal waste products in water. This technique could allow for real-time assessment of water quality without the need for expensive laboratory equipment.

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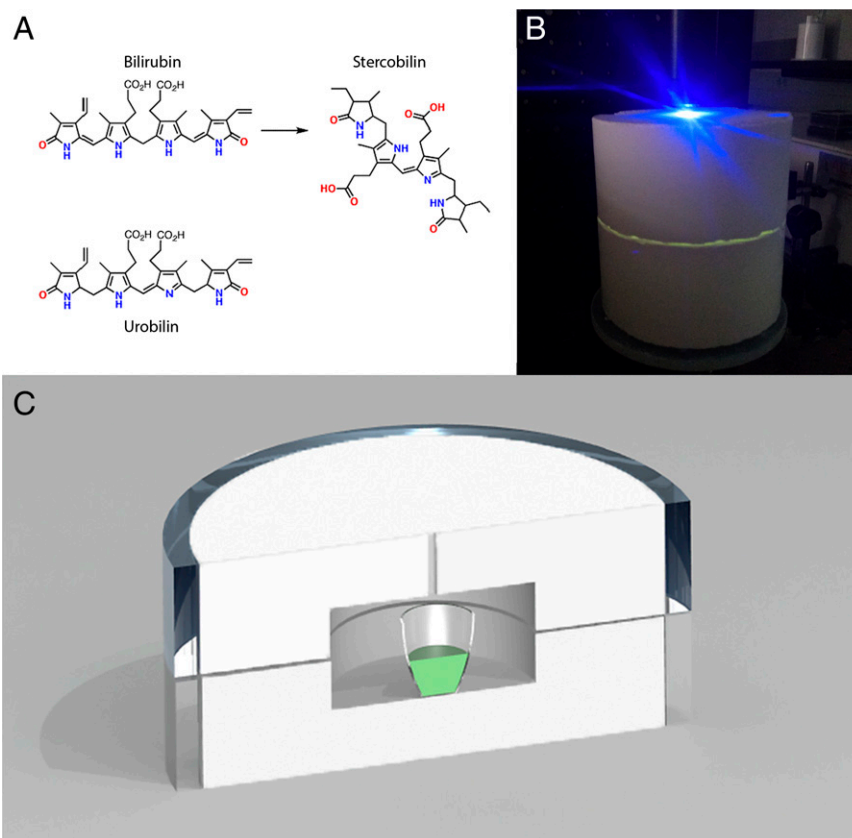


Fig. 2. (A) Chemical structure of bilirubin, stercobilin, and urobilin. These products are generated from hemoglobin metabolism, and are found in human waste products. (B) Photograph of integrating cavity during use. The excitation (blue light) can be seen entering the cavity. The green band visible is the fluorescent emission generated from a high concentration of urobilin in solution. During data acquisition, the cavity halves were clamped together to prevent light loss from this seam. (C) Cross-sectional rendering of the cavity including the crucible used to hold samples.

to indicate the potential for single femtomolar detection, without the need for expensive laser sources. In addition, measurements can be taken in near real time, as integration times below 1 s were sufficient for all samples.

Discussion

In summary, we demonstrate detection of ultralow concentrations of urobilin in solution via the use of an integrating cavity to enhance both the excitation and collection of

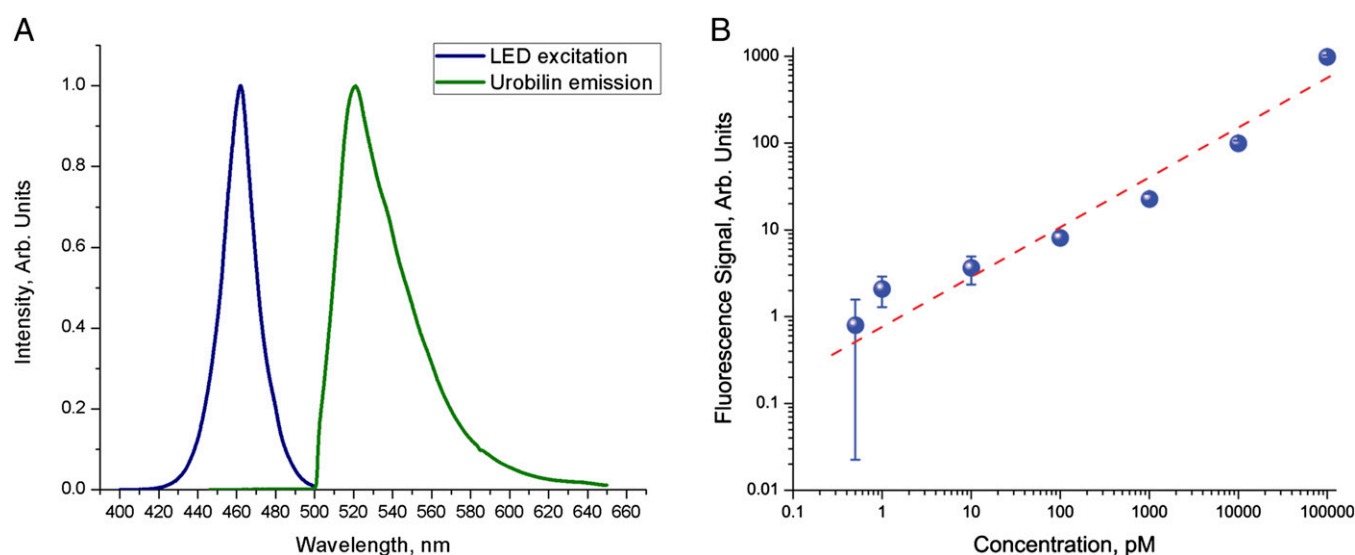


Fig. 3. (A) Excitation and emission spectrums for the LED and urobilin fluorescence. The blue trace shows the LED emission after it was bandpass filtered. The green traces shows the typical fluorescence observed from the cavity. (B) Fluorescence counts plotted against concentration following cavity and ethanol background removal and correction for varying acquisition times on the spectrometer. The blue dots indicate the average fluorescence intensity measured for each concentration where the error bars represent SD between samples. The red dashed trace shows a linear fit to these data, indicating the potential for detection of even lower concentrations.

fluorescence emission from a sample. By placing the sample to be probed into an integrating cavity, isotropic illumination allows for fluorescent signal to be generated from the entire volume. The elastic scattering of the cavity walls limits energy lost inside the cavity, thus allowing for collection of a larger percentage of the diffuse emission. Significant enhancement can be achieved over conventional epillumination system even with the use of an extremely inexpensive excitation source such as a single LED. This technique has tremendous

potential for analysis of global drinking water supplies, particularly in developing nations and following natural disasters, where sophisticated laboratory equipment may not be available.

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